REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The claims have been revised to define the invention with additional clarity. That revisions have been made should not be construed as an indication that Applicants agree with any view expressed by the Examiner. Rather, the revisions are offered merely to advance prosecution and Applicants reserve the right to pursue any deleted subject matter in a continuation application. Claims 19 and 20 have been cancelled.

Claims 19-22, 24-29, 31, 32, 34-36, 39 and 40 stand rejected under 35 USC 112, second paragraph, as allegedly being indefinite. Withdrawal of the rejection is submitted to be in order in view of the above-noted cancellation of claims 19 and 20, amendment of claims 21, 29, 31 and 34-36, and further in view of the comments that follow (which comments are provided on a point by point basis).

1. The claims require the preparation of a genetic library of DNA molecules, each DNA molecule comprising a nucleotide sequence encoding a binding moiety that comprises an amino acid sequence that is a *cis*-acting DNA binding protein. As will be clear from page 7 of the subject application, *cis*-acting proteins, by definition, are proteins that interact with the DNA sequence that encodes them and that establish a covalent linkage to their own template. That being the case, no basis is seen for requiring recitation in the claims of specific structural characteristics and thus no such recitation has been included.

On page 3 of the Action, the Examiner makes reference to an "essential requirement for six His residues for binding to occur". Respectfully, this comment reflects confusion on the part of the Examiner regarding Example 4. In that Example, the six His residues referred to constitute an example of a "display moiety" not a component of the *cis*-acting DNA binding protein. As will be clear from page 51, the stretch of six histidines binds to the Ni column used.

2. Claim 29 as now presented does not include the term "derived".

5. Claim 31 has been revised for purposes of clarity. The Examiner's comment that "the original claim recites for a library, not non-library DNA" does not provide basis for the rejection. Clarification of any such basis is requested so that Applicants can properly respond.

7. See comments that follow regarding claim 36.

10. Claims 19 and 20 have been cancelled.

A. Claim 21 has been revised to more clearly indicate that the peptides and proteins produced are specifically associated with the DNA encoding sequence through covalent binding. This recitation in paragraph "2)" of the claim is consistent with the preamble.

B. Claim 27 depends from claim 21 which requires the presence of an amino acid sequence for display. Claim 27 further limits claim 21 by providing an upper limit of the number of residues in that sequence. Nothing in claim 27 negates the requirement of the elements of claim 21. Thus, basis for the Examiner's confusion is not understood.

C. Claim 31 as presented is believed to be definite. The Examiner is requested to indicate what aspects of the language used are seen to be unclear – the relevance of the Examiner's reference to the language of the original claim is not seen.

D. While claim 34 has been revised to define the invention with additional clarity, basis for the Examiner's comment that the claim comprises two independent processes is not seen. In step (a) of claim 34, a peptide or protein expression library is contacted with a target molecule. The expression library is simply defined in product by process terms (that is by reference to claim 21). No basis for confusion is believed to result from this library definition.

E. Claim 36 requires that contacting of the sample with a molecular probe be effected under conditions such that target-binding moiety can bind target molecule present in the sample selectively. This functional definition of the conditions is all that should be required as no indefiniteness results from its use.

In view of the above, reconsideration is requested.

Claims 19-22, 24-29, 31, 32, 34-36, 39 and 40 stand rejected under 35 USC 103 as allegedly being obvious over Liu et al alone or in view of Mattheakis et al. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

In accordance with the present invention, DNA molecules encoding a *cis*-acting protein (such as P2-A) fused to a display moiety are expressed. The cis activity of the *cis*-acting protein (e.g., P2-A) ensures that the polypeptides produced as a result of that

expression bind faithfully and covalently to the DNA sequence that served as the template for expression.

The resulting protein-DNA complexes (that is, the resulting peptide or protein expression library) can be used for affinity selection protocols against a given target in order to identify individual DNA sequences that encode ligands (that is, the "displayed" amino acid sequence) to which the target binds. A specific embodiment of the invention is shown in attached Figure 1 merely for purposes of exemplification.

Liu et al relates to the construction of individual plasmids containing P2-A wild type, P2-A (Y450D), P2-A (Y454F) or P2-A (Y450D and Y454F). Each P2-A variant is cloned in-frame with a His tag. The expressed polypeptides from these constructs form inclusion bodies from which purified P2-A variants are isolated using the His tags to which they are fused. Biochemical studies are then conducted on purified proteins to understand the catalytic mechanism of P2-A (see attached Figure 2).

In Liu et al, there is no disclosure of the recovery of covalent protein:DNA complexes consisting of the P2-A gene and the expressed P2-A protein. Only the expressed and purified protein is recovered for biochemical analyses (cleavage of single stranded oligouncleotides containing the ori sequence). There is no disclosure of the formation of a library consisting of P2-A fused to a plurality of peptides. There is no suggestion that the cis property of P2A could be exploited for a library screening method.

Mattheakis et al describes modifications of basic polysome display designed to address one of the main limitations of the process, namely the inherent instability of the

polysome complex (basic polysome display is depicted in Figure 3). Mattheakis et al describe the use of tethers that interact directly or indirectly with the polynucleotide template. Double stranded DNA encoding a tether and a plurality of polypeptide sequences is transcribed and translated *in vitro* to produce polysomes consisting of mRNA-stalled ribosomes-expressed polypeptide.

Several subsequent steps are then described to provide further stability to these complexes prior to affinity selection:

1) an RNA binding protein tether binds to the mRNA of the polysome complex (attached Figure 4): or

2) the mRNA is converted to cDNA by reverse transcription allowing a DNA binding protein tether to bind to the cDNA (attached Figure 5); or

3) a tether consisting of a polypeptide tag is bound by a ligand that is directly or indirectly attached to the encoding mRNA (attached Figure 6).

Mattheakis (column 28, line 42) states:

> "The translation conditions selected are suitable for permitting the tether segment of the nascent polypeptide to bind to its encoding poynucleotide before significant dissociation and diffusion of the nascent peptide from the translation complex occurs, and also to reduce binding between complexes. It may be desirable to stall or slow the elongation cycle of ribosomal translocation to increase the probability of forming the linkage between the tether segment and the polynucleotide containing the nascent peptide coding sequence".

The dependence on the polysome display step prior to the exploitation of the tether segments is necessary since without the formation of the polysome complexes (mRNA-

ribosome-nascent polypeptide complexes) the tether segments could interact with templates encoding incorrect polypeptides (i.e., the faithful link between gene and gene-product would be lost).

The cis activity of *cis*-acting DNA binding proteins of the present invention obviates the need to perform polysome display. The cis activity prevents "significant dissociation and diffusion of the nascent peptide from the translation complex" and obviates the need to "stall or slow the elongation cycle of ribosomal translocation to increase the probability of forming the linkage between the tether segment and the polynucleotide containing the nascent peptide coding sequence".

No cis-acting DNA binding proteins are disclosed or contemplated by the Mattheakis et al methods. The Examiner is again reminded that *cis*-acting proteins are proteins that interact with the DNA sequence that encodes them and that establish a covalent linkage to their own template (see page 7 of the disclosure and the claims).

In Mattheakis et al, the DNA that is required for a DNA binding protein tether to be used must be synthesised by reverse transcription following *in vitro* transcription/translation. This is in contrast to the instant invention wherein the DNA that is covalently bound by the *cis*-acting DNA binding protein is the DNA the expression of which results in production of the protein which binds thereto.

The possibility that DNA binding proteins could be used as the tether without the need for a reverse transcription step after the *in vitro* transcription/translation reaction is not taught by, nor would it have been suggested by, Mattheakis et al.

The Examiner appears to be of the view that it would have been obvious to use the *cis*-acting protein of Liu et al in the method of Mattheakis et al. Conspicuous by its absence, however, is any explanation by the Examiner as to where in the art motivation for making such a substitution is found. Applicants submit that in fact no such motivation exists.

As pointed out above, Liu et al does not teach the recovery of covalent DNA:protein complexes and thus is unrelated to the display approach of Mattheakis et al (or of the present invention). Mattheakis et al teaches a fundamentally different display approach than that of the instant claims. Nothing in Mattheakis et al would have suggested totally redesigning that approach to accommodate the *cis*-acting protein of Liu et al. Reconsideration is thus requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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